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COMPARATIVE EVALUATION OF SOME NEW TRANEXAMIC ACID DERIVATIVES AND THEIR COPPER (II) COMPLEXES FOR ANTITUMOR, ANALGESIC, BACTERICIDAL AND FUNGICIDAL ACTIVITIES

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ABSTRACT

The objective of this series of investigation was the synthesis and comparative evaluation of biological and pharmacological activities of some substitutive derivatives of Tranexamic Acid **(T.A)** and their copper (II) complexes. N-Phthaloyltranexamic Acid (**A-1**), N-Acetyltranexamic Acid (**A-2**), Di-Tranexamate Diaquo Copper (II) (**C-1**) Di-N-Phthaloyltranexamate Diaquo Copper (II) (**C-2**) and Di-N-Acetyltranexamate Diaquo Copper (II) (**C-3**) were synthesized, using novel and reproducible procedures. These compounds were characterized by exploiting techniques such as FTIR, Mass Spectroscopy and FT ^{1}H NMR. Bioactivities of the compounds were carried out and it was found that these compounds exhibited excellent anti-tumor, significant analgesic and antifungal activities.

INTRODUCTION

Tranexamic acid (Trans-4-aminomethylcyclohexane carboxylic acid-C_8 H_{15} NO_2) is the derivative of amino acid lysine. This drug inhibits the proteolytic activity of plasmin and the conversion of plasminogen to plasmin by plasminogen activators. It is used for its antiplasminic, hemostatic, antiallergic and anti-inflammatory activities (Anthony J.H. *et al.*, 1988 and Nakanishi A., 1999).

A review of the literature revealed that the substitutive derivatives of some drugs show different and in most of the cases, more effective activities than their parent compounds (Daidone G. *et al.,* 1989; Ahmad B.D. *et al.,* 200 and Pang H *et al.,* 1998). For example it has been found that the O-heterocyclic substituted salicylamides are more effective than salicylamide and in some cases less toxic (Fahmy H.H. and El-Raki W., 2001). Moreover, in our previous work we have found that copper complexes of nonsteroidal anti-inflammatory drugs (NSAID) are more effective than their parent drugs (Khan G.M. *et al*., 1997a and 1997b).

In view of the above findings, interest was emphasized in the investigation to synthesize and characterize some new and novel phthaloyl and acetyl derivatives of Tranexamic acid. Moreover, efforts were also made to synthesize copper (II) complexes of the drug and its derivatives, the results of which have already been published (Khan M.F. *et al.*, 2001). In order to ascertain if the newly synthesized derivatives and/or the copper complexes would offer some better and different activities than their parent compound, comparative evaluation of their biological and pharmacological activities were performed.

MATERIALS AND METHODS

Tranexamic Acid **(T.A)** was a gratis supply from Tabros and Organon Pharmaceuticals (Pvt.) Ltd. N-Phthaloyltranexamic Acid (**A-1**), N-Acetyltranexamic Acid (**A-2**), Di-Tranexamate Diaquo Copper (II) (**C-1**) Di-N-Phthaloyltranexamate Diaquo Copper (II) (**C-2**) and Di-N-Acetyltranexamate Diaquo Copper (II) (**C-3**), synthesized in the previous work (Khan M.F. *et al.,* 2001) of this series of investigation, were used.

In vitro bactericidal, fungicidal, anti-yeast bioassay, effects of the derivatives on blood pressure of anesthetized rats, analgesic activity, *in vitro* phyto-toxic (anti-tumor) activity (potato disc assay) of the samples were carried out at H.E.J. International Research Institute of Chemistry, University of Karachi, Karachi.

Biological Studies

The derivatives and the complexes were checked against various organisms. The procedures used for measuring their activities are described as follows:

In vitro Bactericidal Bioassay

The agar well diffusion method (Kazmi S.U. *et al.*, 1991) was used for carrying out the bactericidal activity test for the derivatives and the complexes. The human pathogens were studied for this purpose. The bacterial zone of inhibition (mm) was measured. The agar medium with standard drugs and complexes, including the pathogens, was incubated for 18 hours at 37^{o}C. Tetracycline was used as a reference drug.

In vitro Fungicidal Bioassay

The fungicidal activity test for the derivatives and the complexes was performed using the agar tube dilution method (Kazmi S.U. *et al.,* 1991). Human, animal and plant pathogens were studied for this purpose. The % linear growth inhibition (mm) was estimated, taking 400 μg of the samples in one ml of the media. The samples were incubated at 37^{o}C for a period of 7 days. Miconazole, Ketoconazole, Amphotericin-B, Flucytosine, Benlate and Nabane were used as standard drugs.

Anti-Yeast Bioassay

The anti-yeast activity test for the derivatives and the complexes was carried out through detection of DNA damaging effects of the agents. The mutant and wild type strains of Saccharomyces cerevisiae were studied for the purpose. The yeast zone of inhibition (mm) was measured using Streptonigrin was used as a reference drug.

PHARMACOLOGICAL STUDIES

Effect of C-1 and C-2 on Blood Pressure and Heart Rate

The effects of the derivatives on blood pressure and heart rate were studied in normotensive anaesthetized male Sprague-Dawely Wistar rats (230-260 g) (Gilani A.H., 1991). The animals were anaesthetized with thiopental sodium (60-80 mg/kg body weight, i.p). The arterial blood pressure was recorded from the carotid artery via the arterial cannula connected to a pressure transducer (Statham P_{23}) coupled with a grass model 79 polygraph. Drugs were injected via the cannula inserted in the jugular vein. Mean blood pressure was calculated as the diastolic blood pressure plus one-third of pulse width. The normal response of the animal was checked through the standard compounds, acetylcholine (hypotensive) and noradrenaline (hypertensive).

Table-1
In vitro bactericidal bioassay

Name of Bacteria	Clinical Implication	Activities of Ligands and Cooper (II) Complexes					Activity of Peference Drug (Tetra-cycline)
		A1	A2	C1	C2	C3	
Human pathogens Bacillus subtilis	Food poisoning	-	-	-	-	-	+++
Corynebacteriun diptheriae	Dipthera, infection of ear, nose, throat and skin. Toximia: Cardio-respiratory failure	-	-	-	-	-	++++
Escherichia coli ETEC	Infections of wounds and urinary tract. Inflammation of Peritoneum and GIT, dysentery, septicemia, neonatal meningitis.	-	-	-	-	-	+++
Klebslella pneumonia	Infections of respiratory and urinary tract. Supportive infections in sinuses and middle ear etc, septicemia	-	-	-	-	-	+++
Proteus vulgaris	Infections of urinary tract and wounds, septicemia	-	-	-	-	-	+++
Pseudomonas aeroginosa	Infections of wounds, urinary tract and eyes. Septicemia.	-	-	-	-	-	++
Salmonella typhi	Typhoid fever, salmonella food poisoning. Localized infection: pyelonephatis, endocarditis, salpingitis, chronic osteomyelitis.	-	-	-	-	-	++++
Shigella dysentery	Inflammation of GIT, bacterial dysentery	-	-	+	-	-	++++
Staphylococcus aureus	Food poisoning, scalded skin syndrome, toxic shock syndrome. Infections of upper respiratory tract and wounds Abacesses, endocarditis.	-	-	-	-	-	+++
Streptococcus pyrogenes	Acute rheumatic fever, scarlet fever, sore throat, orysipelas, aeptic wounds, impetigo, inflammations of post glomerulonephrone (kidney), tonsils and middle ear puerperal sepsis, erythema nodosum.	-	-	-	-	-	++

Key: A1= N-phthaloyltranexamic acid, A2=N-acetyltranexamic acid, C1=Copper complex of Tranexamicd acid, C2=Copper complex of N-phthaloyltranexamic acid, C3=Copper complex of N-acetyltranexamic acid, ++++ = very high activity, +++ = high activity, ++ = optimum activity, + = low activity, - = no activity.

Table 2
In vitro fungicidal bioassay

Name of Fungi	Clinical Implication	Activities of Ligands and Copper (II) complexes					Standard Drugs	Acti-vity
		A1	A2	C1	C2	C3		
Human Pathogens Epidermo-phyton Floccosum	**Cutaneous Mycoses** Ring worm of groins, arms and torso	++	++	++	++	++	Miconazole Ketoconazole	+++++ +++++
Trichophyton Schoenleinii	Scaring of the scalp, permanent alopecia	++	++++	++++	++++	++	Miconazole Ketoconazole	+++++ +++++
Pseudalle-scheria boydii	**Subcutaneous Mycoses** Infection of skin subcutaneous tissue, nasalsinuses, myceto-ma and brainabscess	++++	++	+++	++++	++++	Miconazole Ketoconazole	++++ ++++
Candida albicans	**Opportunistic Mycoses** Candidosis, infection of lungs, vagina, ear, bones, heart and thrush	+	-	-	+	+	Miconazole Ketoconazole	+++++ +++++
Aspergillus niger	Infection of lungs, eyes and CNS. Hypersensitivity and fungal ball	++++	+	++	+	++++	Amphoteri-cin-B Flucytosine	+++++ +++++
Animal Pathogens Microsporum canis	**Cutaneous Mycoses:** Ringworm infection of hair and skin in dogs and cats	+++	++++	++++	++++	++	Miconazole Ketoconazole	+++++ +++++
Trichophyton Mentagro-phytes	Ringworm of feet, nails, fore arms and groins in rodents	+++	++	++	++++	++	Miconazole Ketoconazole	+++++ +++++
Trichophyton sirnii	Sever combined inflammatory and hypersensitivity reaction "kerion" in monkeys, rare (India)	++	++++	+++	++	++++	Miconazole Ketoconazole	+++++ +++++
Plant Pathogens Fusariumum solanivar. Lycopersici (tomato)	**Seed Borne Pathogens:** Root Rot, stemcan-kers of wounds, damping off seedling, destruction of spawn inbeds of cultivated mushrooms and pea crop.	+++	+	++++	++++	+	Benlate Nabam	+++++ +++++
Macropho-mina phaseo-lina	Seed Rot, Wilt, Root rot (Charcoal rot)	++++	+	++	+	++	Benlate Nabam	+++++ +++++
Rhizoctonia Solani	Root Rot (necrosis), wilt.	++	++	++++	++	++	Benlate	+++++

Key: A1=N-phthaloyltranexamic acid, A2 = N-acetyltranexamic acid, C1 = Copper complex of Tranexamic acid, C2 = Copper complex of N-phthaloyltranexamic acid, C3 = Copper complex of N-acetyltarnexamic acid, +++++ = very high activity, ++++ = high activity, +++ = optimum activity, ++ = low activity, + = very low activity, - = no activity. {Incubation Period = 7 days, Incubation Temperature (28 ± 1°C)}

Table 3
Anti-yeast bioassay of Copper (II) Complexes

Name of Yeast	Activities of Copper (II) Complexes					Activity of Streptonigrin (standard drug)
	A-1	A-2	C-1	C-2	C-3	
Saccharomyces cerevisiae (m). RS 322Y (RAD52)	-	+	-	-	-	+++
Saccharomyces cerevisiae (w). LF 15 (RAD_+)	-	-	-	-	-	++

Key: +++ = very high activity, ++ = high activity, + = low activity, - = no activity, m = mutant strain, w = wild type strain.

Table 4
Analgesic activity of the derivatives

Animal used: Male Albino mice (25-30 gm) NMRI strain.
Route of administration: Intraperitoneal (I.P.)
Reference Standard used: Aspirin (Acetyl salicylic acid of chemical grade)

Compound	Dose (mg/kg)	Acetic acid induced writhing response		Remarks
		No. of Writhes	% Inhibition	
Control	-	48	-	Both compounds have shown excellent analgesic activities. The compound F1 inhibited the acetic acid induced writhes in dose dependant manner and at the dose of 100 mg/kg its effect is comparable with aspirin. F2 was found more a potent compound.
Aspirin	150	16	67	
N-phthaloyltran-examic acid (F1)	10	45	6	
	50	22	54	
	100	19	60	
N-acetyltranexamic acid (F2)	10	24	50	

Acetylcholine (1 µg/kg) was used as a positive control which produced 59.5 ± 8.3% fall (mean ± SEM, n=3) in the mean blood pressure (Gilani A.H. 1991).

Analgesic Activity

The analgesic activity test for the derivatives was carried out as follows. The male albino

mice (25-30 g) were randomly divided into several groups. Each mouse was injected intraperitoneally with 1% acetic acid in a volume of 0.lmL/l0g body weight. The symptoms of the acetic acid induced abdominal writhing were similar to those described by Emele (Emele J.F. and Shanaman J., 1963). Five minutes after the injection of acetic acid, the mice were observed for 10 minutes and the number of writhing responses for each mouse was recorded. Aspirin (acetyl salicylic acid of chemical grade) was used as a reference drug.

In vitro Phytotoxic (Anti-Tumor) Bioassay

The anti-tumor activity test (potato disc assay) for derivatives and the copper (II) complexes was performed, using 10 plants of Lemna acquinoctialis welv for the purpose. The highest doses 500 µg per ml of the derivatives and the copper (II) complexes were employed. The sample was incubated at 28±1^{o}C (Rehman A.U., 1999; Rao C.M. *et al.*, 1988, Ferrigni, N.R. *et al.,* 1982 and Pavia D.L. *et al.,* 1979).

Table 5
In vitro phytotoxic (Anti-Tumor) bioassay

Name of Plant	Concentration µg/ml	No. of Fronts		% Growth Regulation	Reference Inhibitor	FI 50* µg/ml
A-1 Cotnrol						
Lemna acquinoctialis Welv	500	00	21	100.00	Praqual 100%	0.000 0.125
	50	14	19	26.31		
	05	16	16	0.0		
A-2 Control						
Lemna acquinoctialis Welv	500	10	21	100	Praqual 100%	0.000 0.125
	50	17	29	100		
	05	19	20	85		
C-1 Control						
Lemna acquinoctialis Welv	500	00	11	100	Praqual 100%	0.000 0.125
	50	00	09	100		
	05	07	09	22		
C-2 Control						
Lemna acquinoctialis Welv	500	00	11	100	Praqual 100%	0.000 0.125
	50	00	9	100		
	05	8	10	20		
C-3 Control						
Lemna acquinoctialis Welv	500	00	11	100	Praqual 100%	0.00 0.125
	50	00	9	100		
	05	2	9	17		

Key: A-1 = N-phthaloyltranexamic acid, A-2 = N-Acetyltranexamic acid, C-1 = Copper complex of Tranexamicd acid, C-2 = Copper complex of N-phthaloytranexamic acid, C-3 Copper complex of N-Acetyltranexamic acid, F150*= Concentration necessary to inhibit and promote 50% of frond prolifration.

RESULTS AND DISCUSSIONS

In vitro Bactericidal Bioassay:

The results of anti bacterial bioassay of the derivatives and the Copper (II) Complexes against human pathogens are given in Table 1. As seen from the table, the derivatives as well as the

complexes showed little activity towards the pathogens. The only exception is that of the **C-1**, which showed low activity against Shigella dyscentry (causative organism of GIT inflammation-bacterial dysentery). It may be due to the presence of copper in the complex. Doxycycline was used as a reference drug.

In vitro Fungicidal Bioassay:

The results of the fungicidal activity tests for the derivatives and Copper (II) Complexes against various fungi are given in Table 2. As seen in the table, the derivatives as well as the Copper (II) Complexes proved themselves to be good fungicides, by inhibiting the growth of various fungi, harmful to human beings, animals and plants.

Anti-Yeast Bioassay:

The anti-yeast activities of the derivatives and the Copper (II) Complexes are shown in Table 3. Except **A-2** and **C-1**, which showed low anti-yeast activities, all the other derivatives as well as Copper (II) Complexes were found to have no anti-yeast activity against mutant strain {RS 322 Y (RAD52)} and wild type strain {LF15 (RAD_+)} of Saccharomyces cerevisiae. Streptonigrin was used as a reference drug.

Effect on Blood Pressure and Heart Rate:

Both, **A-1** and **A-2** at the doses of 10, 25 and 40 mg/kg body weight showed no effect on blood pressure and heart rate of the male Sprague-Dawely rats (230-260g). The normal response of animal was checked through standard compounds such as acetylcholine (Hypotensive) and noradrenaline (Hypertensive).

Analgesic Activity:

The results of the tests performed for knowing the analgesic activities of **A-1** and **A-2** are shown in Table 4. It was found that both of them have excellent analgesic activities. Aspirin was used as a standard drug.

In vitro Phytotoxic Activity:

Table 5 shows the results of the *in vitro* anti-tumor activity test (potato disc assay) for the derivatives and the Copper (II) Complexes. It was found that **A-1** had 100% anti-tumor activity at the dose of 500 µg/ml; **A-2** showed low anti-tumor activity at the dose of 500 µg/ml; while each one of the Copper (II) Complexes exhibited 100% anti-tumor activities at a dose of 500 µg/ml.

CONCLUSIONS

A-1 exhibited excellent anti-tumor, significant anti-fungal and analgesic activities. However, it showed no anti-bacterial and anti-yeast activities. Also, it has no effect on blood pressure and heart rate of anesthetized rats. **A-2** showed excellent analgesic activity, significant anti-fungal and low anti-tumor (phytotoxic) activities. However, it showed no anti-bacterial and anti-yeast activities. Also, it exhibited no effect on blood pressure and heart rate of anesthetized rats.

C-1 exhibited high anti-tumor (phytotoxic) and significant anti-fungal activities. It showed low anti-yeast activity. However, it showed no antibacterial activity. On the other hand, **C-2** and **C-3** showed high anti-tumor (phytotoxic) and significant anti-fungal activities. However they exhibited no anti-yeast and no anti-bacterial activity.

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